Effect of Dynamic Environment on the Interaction between Nanoparticles and Human Airway Epithelial Cell Monolayer

H. Patel*, H. Kim** and S. Kwon***

*Department of Biological Engineering, Utah State University, Logan, UT, USA, hemangpatel31@yahoo.co.in
** Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, UT, hanseup@ece.utah.edu
*** Department of Biological Engineering, Utah State University, Logan, UT, USA, soonjo.kwon@usu.edu

ABSTRACT

Despite their great use for engineering and medical applications, nanomaterials may have adverse consequences upon accidental exposure and medical application due to their nanoscale size, composition and shape. Like many nanomaterials, carbon nanotube (CNT) has been well explored for many proven applications, but very little explored to understand their potential toxic effects. It is crucial to develop viable alternatives to *in vivo* tests to evaluate the toxicity of engineered CNTs. To evaluate the CNT-mediated toxicity in a novel dynamic *in vitro* model, which can simulate normal breathing condition, two different sizes (short: OD 1-2 nm, length 0.5-2 μm; long: OD 1-2 nm, length 5-30μm) of single walled carbon nanotubes (SWCNTs) were used at different concentrations (5, 10, and 20 μg/ml) along with different exposure time (24, 48, and 72 hours) both static and dynamic environments. The dynamic environment facilitated interaction between SWCNTs and A549 monolayer and cellular responses are significantly different from those under static condition. Short SWCNTs decreased reactive oxygen species (ROS) at higher concentration under longer exposure, but cells exposed to long SWCNTs showed significant decrease at all concentrations under longer exposure. Long SWCNTs induced much higher level of IL-8 expression, especially under longer exposure time with all concentrations. The outcome of this study would help us to understand the cellular responses of SWCNT exposure to human airway and the mechanism of progression of inhaled CNTs in the respiratory system.

Keywords: carbon nanotube, nano-toxicity, dynamic culture system, ROS, IL-8.

1 INTRODUCTION

The respiratory system is especially susceptible to insult by airborne toxic materials. Particles that can enter the respiratory system are broken down into three major regions: (1) dust reaching the gas exchange, or alveolar, region is called *respirable dust*; (2) dust reaching the tracheobronchial region and alveolar region is called *thoracic dust*; and (3) dust entering the nose and mouth is called an *inhalable dust*. Respirable dust is smaller than about 4 μm aerodynamic equivalent diameters, thoracic dust is smaller than about 10 μm, and inhalable dust is smaller than about 100 μm. Larger airborne particles can deposit in the upper respiratory system [1]. In a manufacturing environment, CNTs are handled in much larger quantities as compared to typical laboratories, subjecting the workers to a higher risk of exposure to these potentially hazardous nanoparticles. The nanotechnology community in the U.S., led by NIOSH (The National Institute for Occupational Health and Safety) and OSHA (Occupational Safety & Health Administration) is devoting efforts to issue "best practices" guide for safely working with nanomaterials. However, the development is still in its infant stage, and there is a strong need for science-based methodologies to predict the health and toxicological effects of CNTs.

Intensive studies on the toxicity of CNTs have shown that exposure to CNTs results in pulmonary inflammation [2-8]. The inflammatory lung reactions (alveolitis) are a source of genetic lesions which could eventually lead to the development of lung cancer [2]. *In vivo* studies performed using guinea pigs and rats showed the appearance of multifocal granulomas, resulting in inflammatory reactions of the terminal and respiratory bronchials. Mild fibrosis in the alveolar septa was also observed [9]. Ken Donaldson and his colleagues described three properties of CNTs associated with pathogenicity in particles. They are 1) nanoparticles showing more toxicity than larger sized particles, 2) fiber-shaped particles behaving like asbestos and other pathogenic fibers which have toxicity associated with their needle-like shape, and 3) biologically biopersistent. They also pointed out that CNTs are possibly one of the least biodegradable man-made materials ever devised [10]. Also concerns over the increased emissions of CNTs into the environmental compartments (air, water and soil) mainly due to improper disposal of CNTs were raised [9]. Recent studies for nanomaterials indicate: (1) CNTs and fullerenes have produced toxic effects on biological systems [9,11-14]; (2) evidence that nanoparticles can translocate to bloodstream [15, 16]; and

(3) evidence that nanoparticles can cross blood brain barrier [17]. However, studies are still preliminary, as the current *in vivo* and *in vitro* response data are difficult to extrapolate.

The airway wall exists in a mechanically dynamic environment, where different amounts of circumferential and longitudinal expansion and contraction occurred during breathing movements. In this study, we established *in vitro* dynamic culture system simulating normal breathing condition of our airway. This dynamic culture system of human airway epithelial cells was used for the investigation of the effect of different size of SWCNTs on cell proliferation, cellular inflammatory response, and the level of reactive oxygen species. The different level of biological and toxicological effects was observed both in static and dynamic conditions of airway epithelial cell monolayer.

2 MATERIALS AND METHODS

2.1 Exposure of SWCNT to A549 monolayers

Following confluency, A549 monolayers were treated with different concentrations (5, 10, and 20 µg/ml) of short and long SWCNTs in F-12k medium for 24, 48, and 72 hours either in static or dynamic condition. To simulate dynamic condition cell monolayers were grown under 5% equibiaxial elongation at 0.2 Hz for the entire duration of exposure using Flexcell 4000T plus system[2]. The levels of pro-inflammatory, oxidative stress and cytotoxic mediators were monitored, following exposure of SWCNTs with different concentration, exposure time, and size.

2.2 Cyclic equibiaxial deformation

We used a physiologically relevant range of cyclic equibiaxial deformation (5%), which corresponds to 45% of the total lung capacity [18]. Flexcell® Tension Plus™ 4000T system (Flexcell International, PA) was used to equibiaxially elongate the monolayers of cells on silicone rubber bottoms of a BioFlex plate. The cell monolayers were exposed to 5% of cyclic equibiaxial deformation for 24 and 48 hours at frequency of 0.2Hz. The 0 hour time refers to the starting point of cyclic equibiaxial deformation.

2.3 Total protein measurement

Total protein concentration from cell lysate was measure using BCA total protein assay (Thermo Scientific, IL) to quantify the amount of A549 cells.

2.4 IL8 measurement

IL8 secretion in media supernatant was measured using ELISA kit (Invitrogen, CA) to check the level of inflammation in A549 cells.

2.5 Reactive Oxygen Species measurement

Reactive oxygen species (ROS) level in media supernatant was measured using 2',7'-dichlorofluorescin (EMD, NJ).

3 RESULTS

Effect of short SWCNT on A549 cell growth

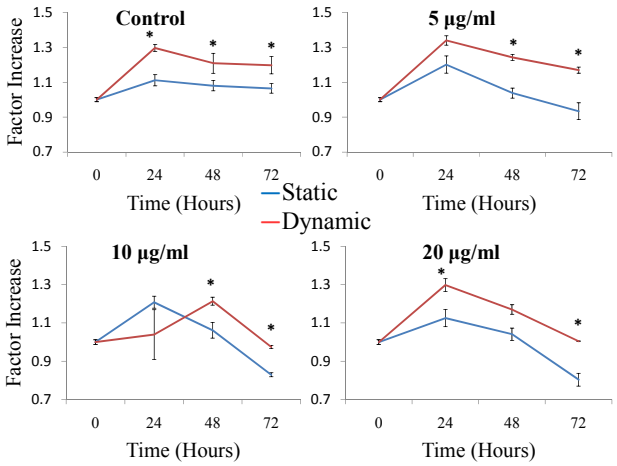

Figure 1. The effect of short SWCNT exposure on A549 cell growth. * significantly different than respective time point of the same exposure level under different cell growth condition ($p<0.05$).

Under both cell growth conditions, A549 cell growth showed increasing trend up to 24 hours followed by decrease for 48 and 72 hour exposure time. The A549 cell growth was significantly higher for all concentrations at 72 hours under dynamic cell growth condition.

Effect of long SWCNT on A549 cell growth

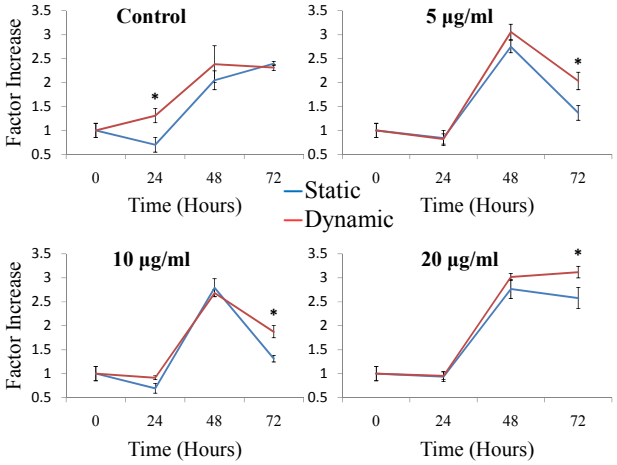

Figure 2. The effect of long SWCNT exposure on A549 cell growth. * significantly different than same exposure

time and concentration under different cell growth condition ($p<0.05$).

After 72 hours of exposure, A549 cell growth showed decreasing trend for both cell growth conditions. However, dynamic cell growth condition showed significantly higher cell growth than that in static condition.

Effect of short SWCNT exposure on ROS production

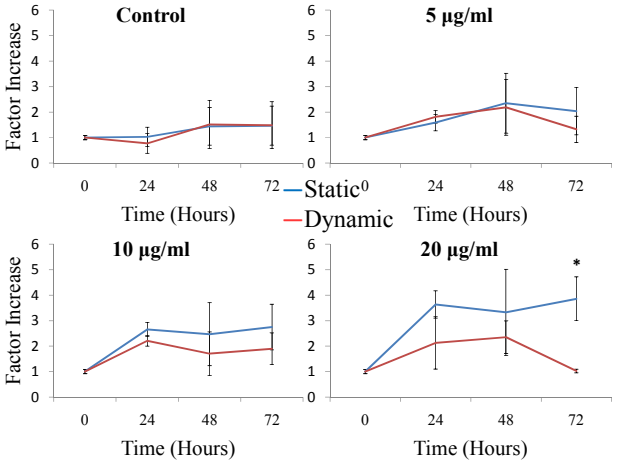

Figure 3. The effect of short SWCNT exposure on ROS production by A549 cell. * significantly different than respective time point of the same exposure level under different cell growth condition ($p<0.05$).

The ROS production was not significantly different for all exposure levels and time points between static and dynamic conditions. Only at 20 µg/ml and 72 hours, significantly higher level of ROS production was observed.

Effect of long SWCNT exposure on ROS production

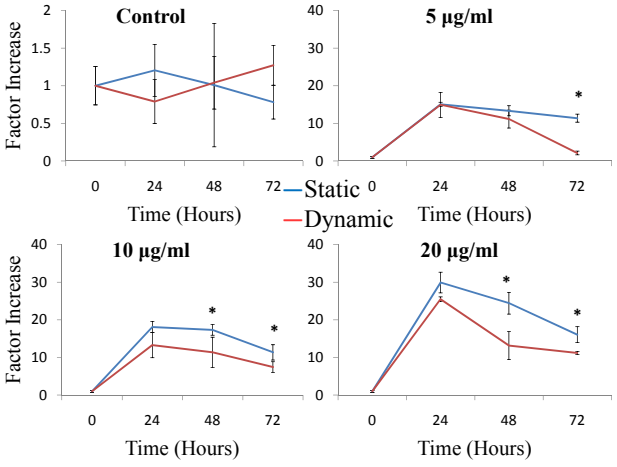

Figure 4. The effect of long SWCNT exposure on ROS production by A549 cell. * significantly different than respective time point of the same exposure level under different cell growth condition ($p<0.05$).

For all concentrations, ROS level was significantly higher for longer exposure times (i.e. 48 and 72 hours) under static growth condition ($p<0.05$).

Effect of short SWCNT exposure on IL-8 expression

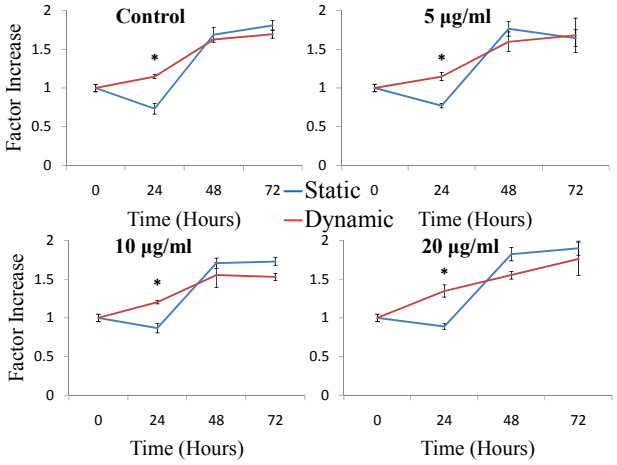

Figure 5. The effect of short SWCNT exposure on IL-8 expression by A549 cell. *significantly different than respective time point of the same exposure level under different cell growth condition ($p<0.05$).

The IL-8 expression was not significantly changed for all exposure levels and time points between static and dynamic conditions. Only for 24 hours time point, dynamic cell growth condition showed significantly higher level of IL-8 expression.

Effect of long SWCNT exposure on IL-8 expression

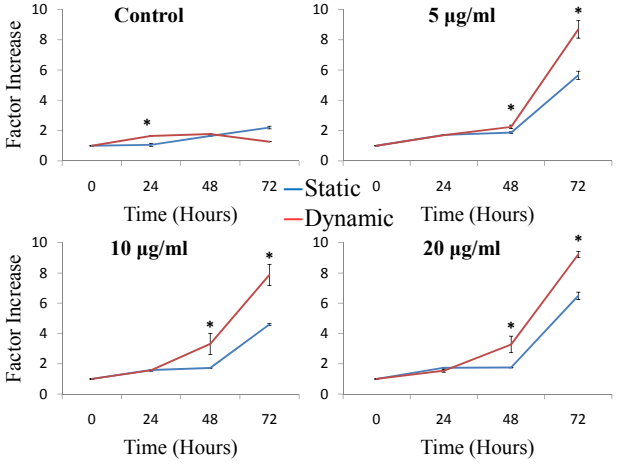

Figure 6. The effect of long SWCNT exposure on IL-8 expression by A549 cell. *significantly different than respective time point of the same exposure level under different cell growth condition ($p<0.05$).

For longer exposure time (i.e. 48 and 72 hours), IL-8 expression was significantly higher for dynamic cell growth condition at all exposure levels ($p<0.05$).

4 DISCUSSION

Although nanomaterials have enormous biological and medical potentials, they may induce potential risk to human health. However, up to now, more fundamental investigation is still required to draw a definite conclusion about the toxicity and safety of any existing nanomaterials. Toxicity of nanomaterials should be assessed in a variety of viable alternative ways, either in static or dynamic environments, to set up the strategy for the safe applications of them to new fields. Especially, we need to consider several important factors such as size, shape, phase, and dose, etc., since any of which either the biological or toxicological effects largely depend on. Especially, CNTs have high aspect ratio in structure and may induce more curvature effects under dynamic environments in our body. We observed that the dynamic environment facilitate interaction between SWCNTs and A549 monolayer and cellular responses are significantly different from those under static condition. In this study, we established *in vitro* dynamic culture system simulating normal breathing condition of our airway. This dynamic culture system of human airway epithelial cells was used for the investigation of the effect of different sizes and concentrations of SWCNTs on cell proliferation, cellular inflammatory response, and the level of reactive oxygen species.

ACKNOWLEDGMENTS

Funding for this study was provided by VPR-RC (Vice President for Research, Research Catalyst Grant) in the Utah State University.

REFERENCES

1. Baron, P.A., A.D. Maynard, and M. Foley, *Evaluation of Aerosol Release During the Handling of Unrefined Single Walled Carbon Nanotube Materials.* Carbon nanotube aerosol generation, 2003. **NIOSH DART-02-191 Rev. 1.1**: p. 1-22.
2. Chou, C.C., et al., *Single-walled carbon nanotubes can induce pulmonary injury in mouse model.* Nano Lett, 2008. **8**(2): p. 437-45.
3. Shvedova, A.A., et al., *Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice.* Am J Physiol Lung Cell Mol Physiol, 2005. **289**(5): p. L698-708.
4. Warheit, D.B., et al., *Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats.* Toxicol Sci, 2004. **77**(1): p. 117-25.
5. Muller, J., et al., *Respiratory toxicity of multi-wall carbon nanotubes.* Toxicol Appl Pharmacol, 2005. **207**(3): p. 221-31.
6. Mitchell, L.A., et al., *Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes.* Toxicol Sci, 2007. **100**(1): p. 203-14.
7. Lam, C.W., et al., *Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation.* Toxicol Sci, 2004. **77**(1): p. 126-34.
8. Li, Z., et al., *Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes.* Environ Health Perspect, 2007. **115**(3): p. 377-82.
9. Helland, A., et al., *Reviewing the environmental and human health knowledge base of carbon nanotubes.* Environ Health Perspect, 2007. **115**(8): p. 1125-31.
10. Donaldson, K., et al., *Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety.* Toxicol Sci, 2006. **92**(1): p. 5-22.
11. Chin, S.F., et al., *Amphiphilic helical peptide enhances the uptake of single-walled carbon nanotubes by living cells.* Exp Biol Med (Maywood), 2007. **232**(9): p. 1236-44.
12. Dumortier, H., et al., *Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells.* Nano Lett, 2006. **6**(7): p. 1522-8.
13. Lam, C.W., et al., *A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks.* Crit Rev Toxicol, 2006. **36**(3): p. 189-217.
14. Yang, K., et al., *Competitive sorption of pyrene, phenanthrene, and naphthalene on multiwalled carbon nanotubes.* Environ Sci Technol, 2006. **40**(18): p. 5804-10.
15. Rothen-Rutishauser, B., et al., *Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model.* Fibre Toxicol, 2007. **4**: p. 9.
16. Shimada, A., et al., *Translocation pathway of the intratracheally instilled ultrafine particles from the lung into the blood circulation in the mouse.* Toxicol Pathol, 2006. **34**(7): p. 949-57.
17. Kim, H.R., et al., *Translocation of poly(ethylene glycol-co-hexadecyl)cyanoacrylate nanoparticles into rat brain endothelial cells: role of apolipoproteins in receptor-mediated endocytosis.* Biomacromolecules, 2007. **8**(3): p. 793-9.
18. Tschumperlin, D.J. and S.S. Margulies, *Equibiaxial deformation-induced injury of alveolar epithelial cells in vitro.* Am J Physiol, 1998. **275**(6 Pt 1): p. L1173-83.